

AMENDMENTS TO THE CLAIMS

Listing of Claims

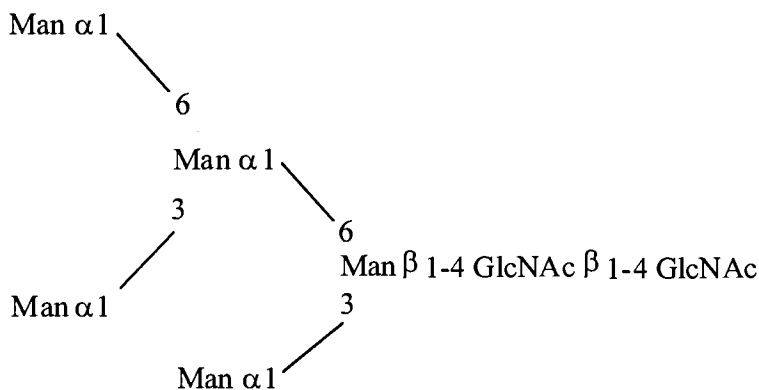
1. (Currently Amended) A process for producing a methylotrophic yeast that produces a mammalian type sugar chain, which comprises the steps of:

- 1) disrupting an *OCH1* gene which encodes α -1,6-mannosyl transferase and YPS1 gene which encodes Aspartic protease 3, in a methylotrophic yeast; and
- 2) introducing an α -1,2-mannosidase gene into the yeast and expressing it therein,

wherein the methylotrophic yeast belongs to the genus *Pichia* or *Ogataea*.

2. (Previously Presented) A process according to claim 1, wherein the mammalian type sugar chain is represented by the following structural formula (Man₅GlcNAc₂):

Structural Formula 2



3. (Canceled)

4. (Original) A process according to claim 1 or 2, wherein the methylotrophic yeast is *Ogataea minuta*.

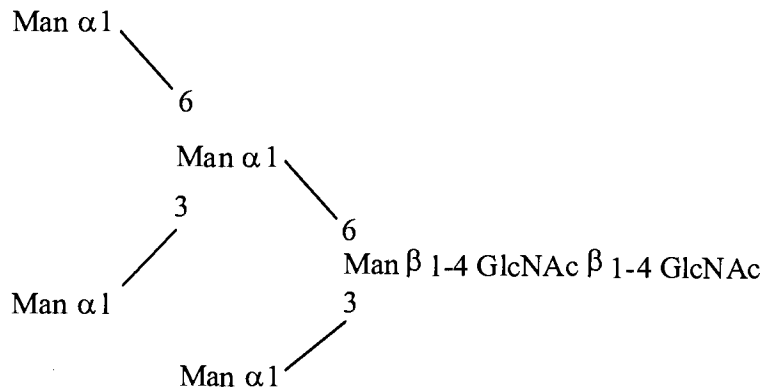
5. (Original) A process according to claim 1, wherein the methylotrophic yeast is a strain from *Ogataea minuta* strain IFO 10746.

6. (Original) A process according to claim 1, wherein the α -1,2-mannosidase gene is expressed under the control of a methanol-inducible promoter.
7. (Original) A process according to claim 6, wherein the methanol-inducible promoter is a promoter of an alcohol oxidase (*AOX*) gene.
8. (Original) A process according to claim 7, wherein the alcohol oxidase (*AOX*) gene is from *Ogataea minuta*.
9. (Previously Presented) A process according to claim 1, characterized in that the α -1,2-mannosidase gene to be introduced is attached to a yeast endoplasmic reticulum (ER) retention signal (HDEL) (SEQ ID NO: 121).
10. (Original) A process according to claim 1, wherein the α -1,2-mannosidase gene is from *Aspergillus saitoi*.
11. (Original) A process according to claim 1, which further comprises a step of transforming a heterologous gene into the yeast.
12. (Original) A process according to claim 11, wherein the heterologous gene is transferred using an expression vector and is expressed in the yeast.
13. (Original) A process according to claim 12, wherein the expression vector comprises a methanol-inducible promoter.
14. (Original) A process according to claim 13, wherein the methanol-inducible promoter is a promoter of an alcohol oxidase (*AOX*) gene.
15. (Original) A process according to claim 14, wherein the alcohol oxidase (*AOX*) gene is from *Ogataea minuta*.
16. (Original) A process according to claim 12, wherein the expression vector comprises a promoter of a glyceraldehyde-3 -phosphate dehydrogenase (*GAPDH*) gene.

17. (Original) A process according to any one of claims 11 to 16, wherein 20% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
18. (Original) A process according to any one of claims 11 to 16, wherein 40% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
19. (Original) A process according to any one of claims 11 to 16, wherein 60% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
20. (Original) A process according to any one of claims 11 to 16, wherein 80% or more of N-linked sugar chains on the protein encoded by the heterologous gene is the mammalian type sugar chain represented by Structural Formula 2.
21. (Original) A process according to any one of claims 11 to 16, wherein the protein encoded by the heterologous gene is from humans.
22. (Original) A process according to any one of claims 11 to 16, wherein the protein encoded by the heterologous gene is an antibody or a fragment thereof.
23. (Original) A methylotrophic yeast produced by a process according to claim 1.
24. (Original) A process for producing a protein encoded by a heterologous gene, wherein the process comprises culturing the methylotrophic yeast of claim 23 in a medium to obtain the protein encoded by the heterologous gene comprising a mammalian type sugar chain from the culture.
- 25.–93. (Canceled)

94. (Previously Presented) A process for producing an *Ogataea minuta* strain, which produces a mammalian type sugar chain represented by the following structural formula (Man₅GlcNAc₂):

Structural Formula 2



comprising a step of disrupting *OCH1* gene (SEQ ID NO:42) in the *Ogataea minuta* strain; and a step of disrupting a *YPS1* gene (SEQ ID NO:115) in the same strain.

95. (Original) A process of claim 94, wherein the *Ogataea minuta* strain is from the strain IFO 10746.

96. (Previously Presented) A process according to claim 94, which further comprises a step of disrupting at least one gene selected from the group consisting of a *URA3* gene comprising the nucleotide sequence represented by SEQ ID NO:15, an *ADE1* gene comprising the nucleotide sequence represented by SEQ ID NO:27, an *HIS3* gene comprising the nucleotide sequence represented by SEQ ID NO:99, and a *LEU2* gene comprising the nucleotide sequence represented by SEQ ID NO:107.

97. (Previously Presented) A process according to claim 94, which further comprises a step of disrupting at least one gene selected from the group consisting of a *PEP4* gene comprising the nucleotide sequence represented by SEQ ID NO:51, a *PRB1* gene comprising the nucleotide sequence represented by SEQ ID NO:57.

98. (Original) A process according to claim 97, which further comprises a step of disrupting a *KTR1* gene comprising the nucleotide sequence represented by SEQ ID NO:63 and/or an *MNN9* gene comprising the sequence represented by SEQ ID NO:69.

99. (Original) A process according to any one of claims 94 to 98, which further comprises a step of introducing and expressing an α -1,2-mannosidase gene from *Aspergillus saitoi*.

100. (Original) A process according to claim 99, wherein the α -1,2-mannosidase gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

(a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;

(b) the α -1,2-mannosidase gene; and

(c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

101. (Original) A process according to claim 94, which further comprises a step of introducing and expressing a *PDI* gene.

102. (Original) A process according to claim 101, wherein the *PDI* gene is a gene (M62815) from *Saccharomyces cerevisiae*.

103. (Original) A process according to claim 102, wherein the *PDI* gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

(a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;

(b) the *PDI* gene; and

(c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

104. (Original) A process according claim 94, which further comprises a step of introducing and expressing a heterologous gene.

105. (Original) A process according to claim 104, wherein the heterologous gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

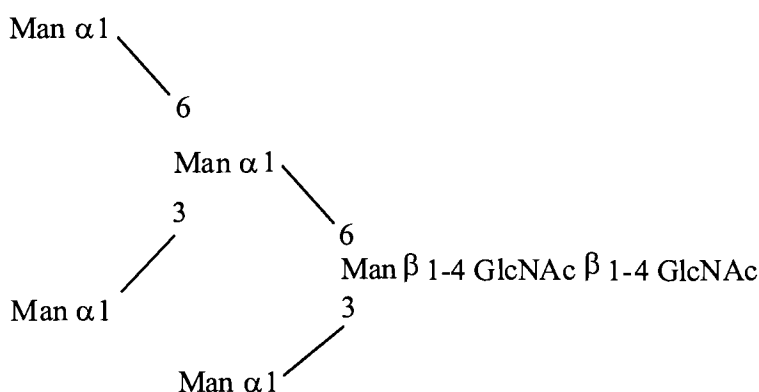
- (a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;
- (b) the heterologous gene; and
- (c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

106. (Original) A process for producing a protein encoded by a heterologous gene, which comprises culturing *Ogataea minuta* produced by the process of claim 104 in a medium, to obtain the protein comprising a mammalian type sugar chain encoded by the heterologous gene from the culture.

107. (Canceled)

108. (Previously Presented) A process for producing an *Ogataea minuta* strain, which produces a mammalian type sugar chain represented by the following structural formula ($\text{Man}_5\text{GlcNAc}_2$):

Structural Formula 2



wherein the process comprises the steps of:

disrupting an *OCH1* gene comprising the nucleotide sequence represented by SEQ ID NO:42 in an *Ogataea minuta* strain; and

disrupting a *URA3* gene comprising the nucleotide sequence represented by SEQ ID NO:15 in the same strain; and

disrupting a *PEP4* gene comprising the nucleotide sequence represented by SEQ ID NO:51 in the same strain; and

disrupting a *PRB1* gene comprising the nucleotide sequence represented by SEQ ID NO:57 in the same strain; and

disrupting a *YPS1* gene comprising the nucleotide sequence represented by SEQ ID NO:115 in the same strain.

109. (Original) A process according to claim 108, wherein the *Ogataea minuta* strain is from the strain IFO 10746.

110. (Original) A process according to claim 108 or 109, which further comprises a step of disrupting an *ADE1* gene comprising the nucleotide sequence represented by SEQ ID NO:27.

111. (Original) A process according to claim 110, which further comprises a step of disrupting a *KTR1* gene comprising the nucleotide sequence represented by SEQ ID NO:63.

112. (Original) A process according to claim 111, which further comprises a step of disrupting an *HIS3* gene comprising the nucleotide sequence represented by SEQ ID NO:99.

113. (Original) A process according to claim 111, which further comprises a step of disrupting a *LEU2* gene comprising the nucleotide sequence represented by SEQ ID NO:107.

114. (Canceled)

115. (Previously Presented) A process according claim 108, which further comprises a step of introducing and expressing an α -1,2-mannosidase gene.

116. (Previously Presented) A process according to claim 115, wherein the α -1,2-mannosidase gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

(a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;

(b) the α -1,2-mannosidase gene; and

(c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

117. (Previously Presented) A process according to claim 108, which further comprises a step of introducing and expressing a *PDI* gene (M62815).

118. (Previously Presented) A process according to claim 117, wherein the *PDI* gene (M62815) is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

(a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;

(b) the *PDI* gene; and

(c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

119. (Previously Presented) A process according to claim 108, which further comprises a step of introducing and expressing a heterologous gene.

120. (Previously Presented) A process according to claim 119, wherein the heterologous gene is expressed from a recombinant expression vector comprising a gene expression cassette comprising:

(a) a DNA comprising a promoter of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:79;

(b) the heterologous gene; and

(c) a terminator of alcohol oxidase (*AOX*) gene which is substantially represented by SEQ ID NO:80.

121. (Previously Presented) A process for producing a protein encoded by a heterologous gene comprising a mammalian type sugar chain, wherein the process comprises culturing

Ogataea minuta produced by the process of claim 119 in a medium to obtain the protein from the culture.

122. (Canceled)